SUPPLEMENTARY MATERIAL ONLINE

Enhancement of the RAD51 Recombinase Activity by the Tumor Suppressor PALB2

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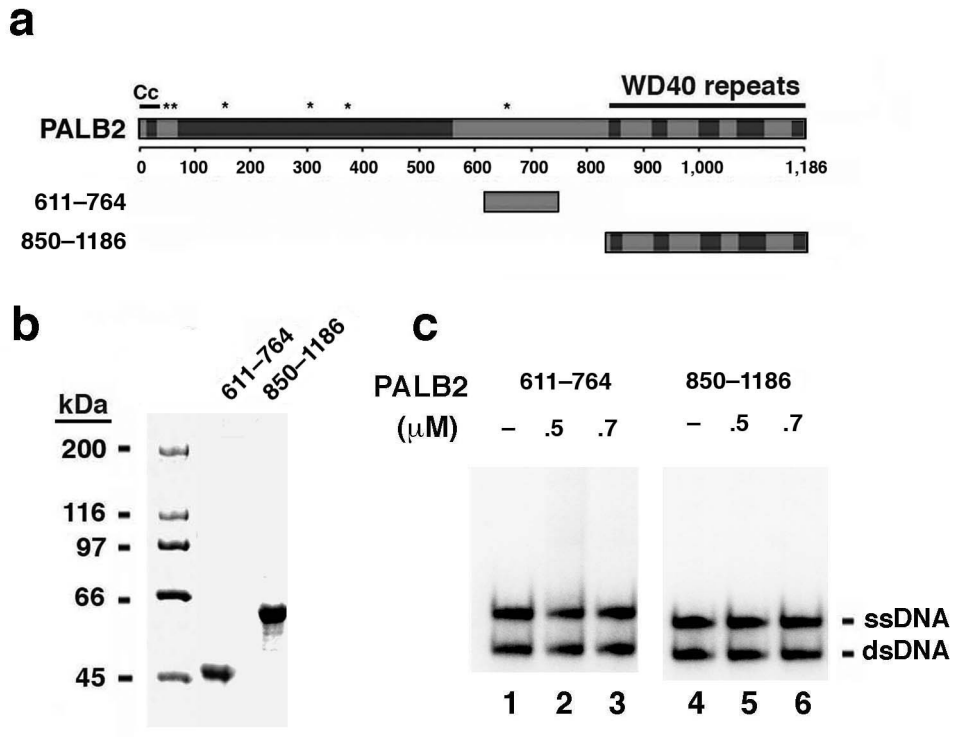
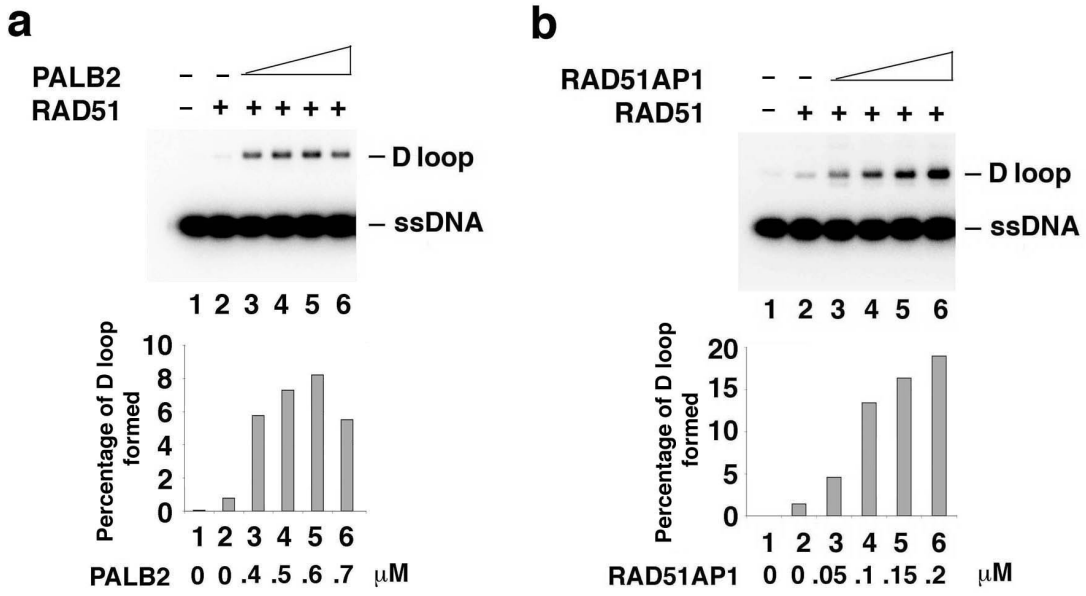
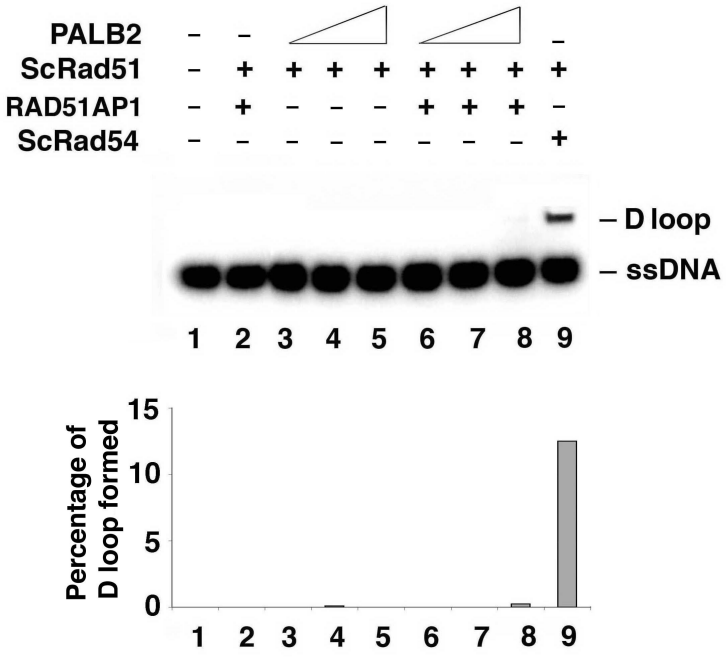


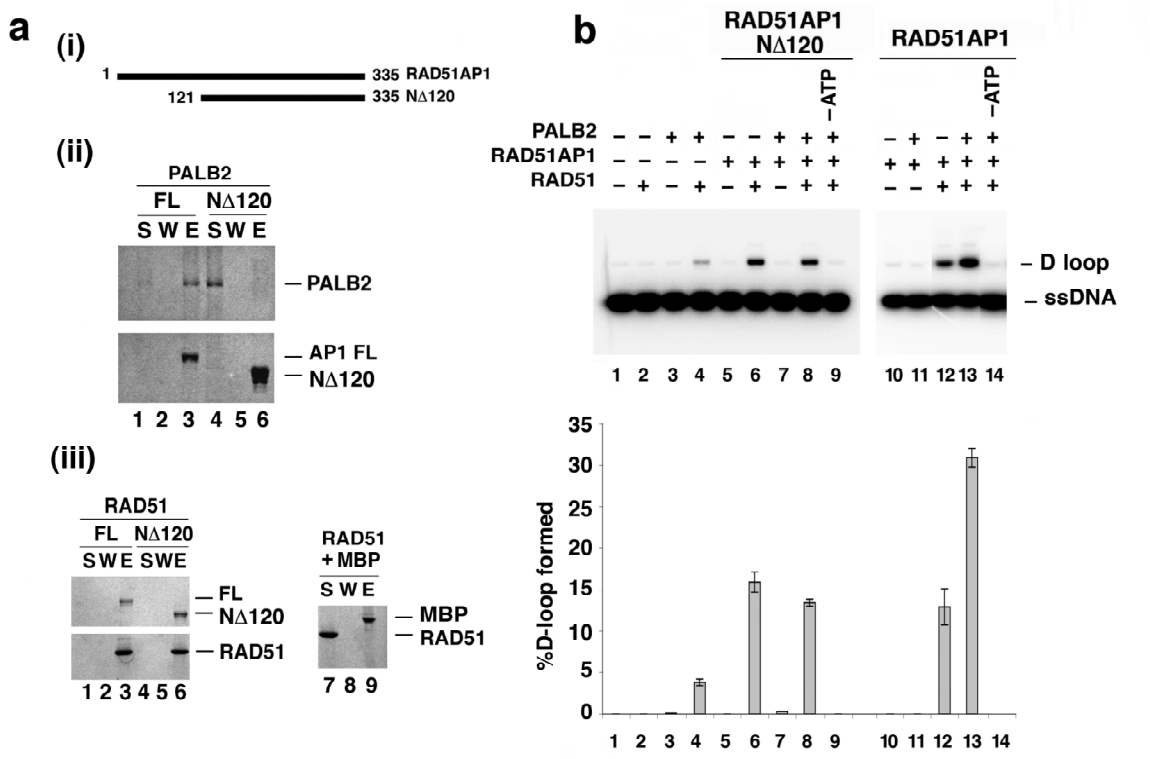
Supplementary Fig. 1. Analysis of PALB2 fragments for DNA binding activity. (a) Schematic summarizing the known features of PALB2 and the PALB2 species used **in** the DNA binding experiment. **(b)** SDS-PAGE analysis of the purified PALB2 species (4 μg each). **(c)** The indicated amounts of PALB2 611-764 or 850-1186 were incubated with a mixture of ssDNA and dsDNA (15 nM each) then analyzed as in Fig. 2.



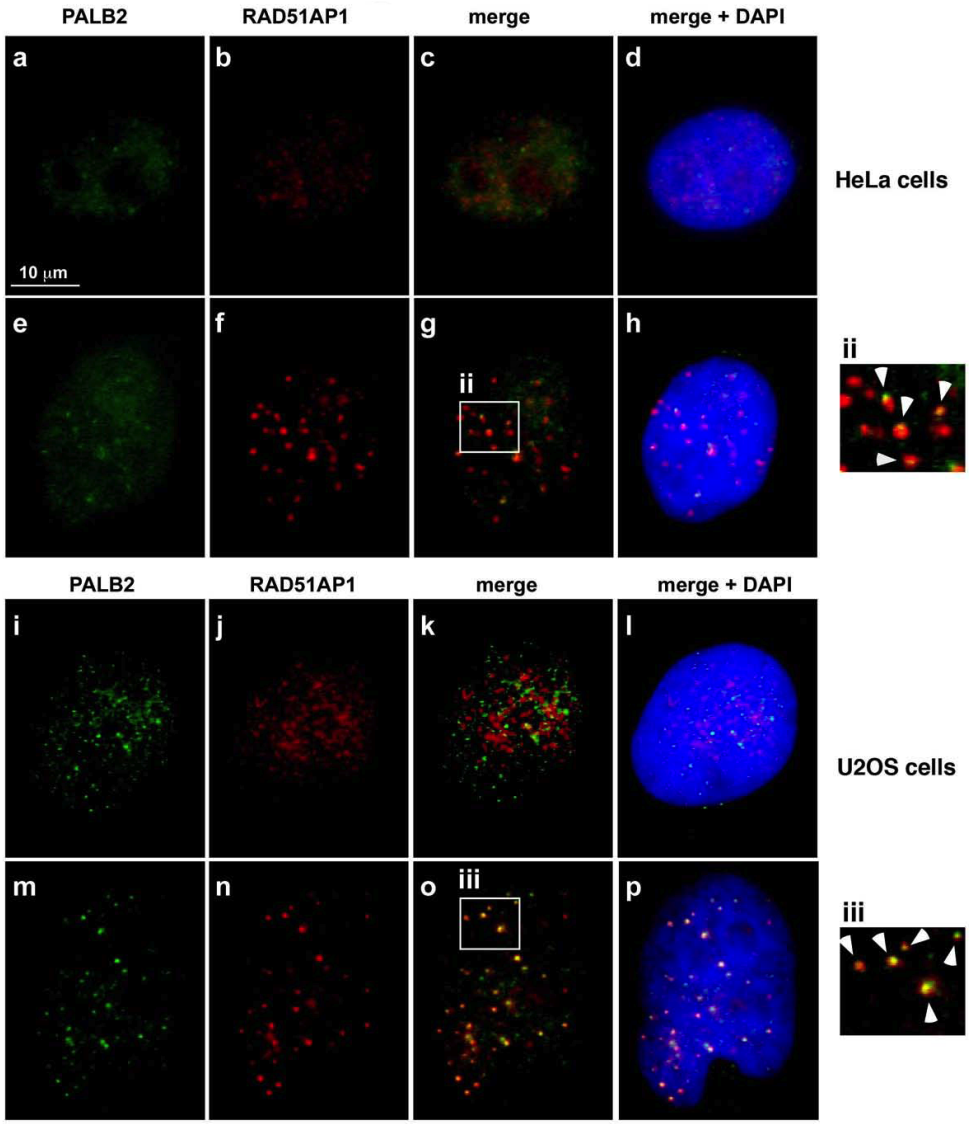
Supplementary Fig. 2. Enhancement of the D-loop reaction by PALB2 or RAD51AP1. An increasing amount of **(a)** PALB2 (400 to 700 nM) or **(b)** RAD51AP1 (50 to 200nM) was examined for the ability to enhance the RAD51-mediated D-loop reaction, as in **Fig. 3b**[1].



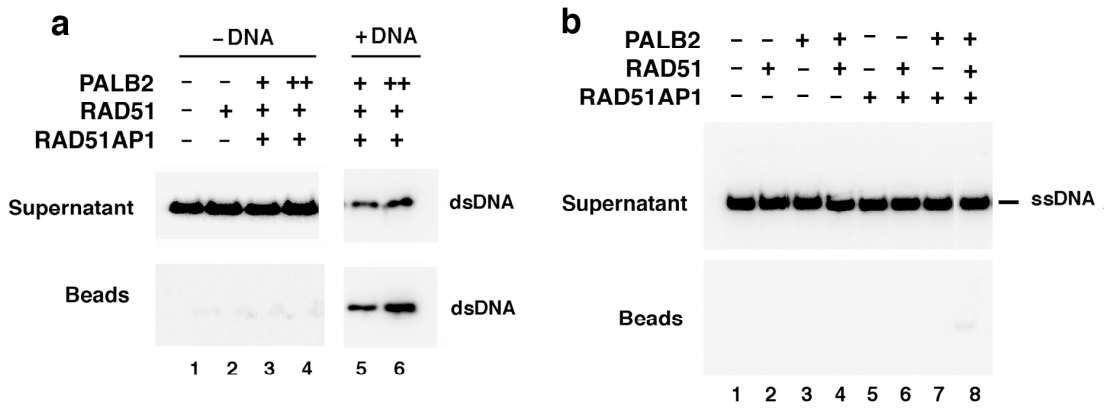
Supplementary Fig. 3. The effect of PALB2 and RAD51AP1 is species specific. We conducted a series of D-loop reactions, as described in Figure 3B, in which RAD51 was replaced with ScRad51. The results showed that neither PALB2 (200, 400, and 600 nM), nor RAD51AP1 (200nM), nor the combination of both factors has any effect on the ScRad51 recombinase activity. ScRad54 (200 nM) was used in conjunction with ScRad51 to provide a positive control[2].



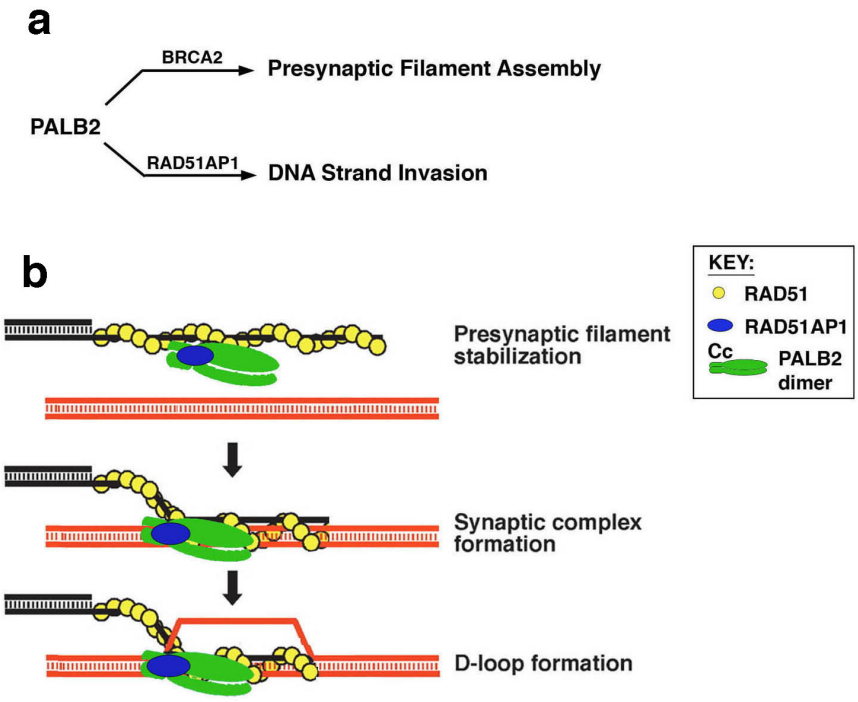
Supplementary Fig. 4. Functional synergy between PALB2 and RAD51AP1 depends on their physical interaction. (a) MBP-tagged RAD51AP1 (2 μg) or RAD51AP1 NΔ120 (4 μg) was incubated with PALB2 (2 μg), and amylose resin was used to capture any protein complex formed, followed by elution of the bound proteins with SDS and SDS-PAGE analysis, as in Figure 3C. **(b)** RAD51AP1 (100 nM) and RAD51AP1 NΔ120 (100nM) were examined for the ability to enhance the RAD51-mediated D-loop reaction with or without PALB2, as in **Fig. 3b**.



Supplementary Figure 5. PALB2 and RAD51AP1 foci co-localize after DNA damage. Shown are representative micrographs obtained for HeLa (**a-h**) and U2OS nuclei (**i-p**). Without DNA damaging treatment, PALB2 (green) and RAD51AP1 (red) are mostly dispersed throughout the nucleus in both HeLa (**a, b**) and U2OS cells (**i, j**) and little or no co-localization was seen (**c** and **k** for HeLa and U2OS cells, respectively). After exposure to 15 Gy X-rays, PALB2 and RAD51AP1 re-localized into distinct foci in both HeLa (**e, f**) and U2OS (**m, n**) cells. A significant fraction of these DNA damage-induced PALB2 and RAD51AP1 foci co-localize (yellow), as depicted by superimposing the individual signals, and specifically highlighted by the arrow heads in the zoomed-in displays (**ii, iii**) of the two indicated regions in panels **g** and **o**. Co-localization of PALB2 and RAD51AP1 foci also is apparent when adding the DAPI channel (**h, p**). In these experiments, a 16-h post-irradiation time point was picked for analysis because the PALB2 foci are more prominent at this time than earlier[3], thus permitting a more facile demonstration of protein co-localization.



Supplementary Fig. 6. The RAD51 presynaptic filament is indispensable for duplex capture and is unable to capture ssDNA. (a) Capture of the duplex DNA requires the presence of ssDNA and hence the presynaptic filaments on the magnetic beads. **(b)** The PALB2-RAD51AP1-RAD51 presynaptic filament ensemble could not capture radiolabeled ssDNA. The concentration of the radiolabeled ssDNA was 4 μM nucleotides and the other reaction conditions were as in **Fig. 4a**.



Supplementary Fig. 7. Multi-faceted role of PALB2 in RAD51-dependent homologous recombination. (a) The BRCA2-PALB2 complex functions in the delivery of RAD51 to chromosomal lesions to initiate their repair[3]. PALB2 also acts in conjunction with RAD51AP1 to enhance RAD51's ability to catalyze D-loop formation (this work). **(b)** Mechanistic model depicting the co-operative action of PALB2 and RAD51AP1 in the RAD51-catalyzed D-loop reaction.

P1	5'-TTATATCCTTTACTTTGAATTCTATGTTTAACCTTTTACTTATTTTGTATTAGCCGGATCCTTATTTCAATTATGTTCAT-3'
P2	5'-ATGAACATAATTGAAATAAGGATCCGGCTAATACAAAATAAGTAAAAGGTTAAACATAGAATTCAAAGTAAAGGATATAA-3'
A1	5'-CATTGCATATTTAAAACATGTTGGAAGGCTCGATGCATGCTGATAGCCTACTAGTGCTGCTGGCTTTCAAATGACCTCTTATCAAGTGAC-3'
A2	5'-GTCACTTGATAAGAGGTCATTTGAATTCATGGCTTAGAGCTTAATTGCTGAATCTGGTGCTGGGATCCAACATGTTTTAAATATGCAATG-3'
A3	5'-CTGCTACGATGCTAGTCGTAGCTCGGCAGTCGTAGCAGGTTCCCAGCACCAGATTCAGCAATTAAGCTCTAAGCCATGAA-3'
SspI	5'-AATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATT-3'
AflIII	5'-CAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGCCAGGA-3'

Table S1: Oligonucleotides used.

SUPPLEMENTARY METHODS

Plasmid construction

The cDNAs for full length PALB2, PALB2 1-579, PALB2 611-764, and PALB2 850-1186 were introduced into the pDEST20 vector (Invitrogen) to fuse them to the GST coding sequence for bacmid production in *E. coli*. The PALB2 fragments encompassing residues 1-100, 101-184, and 1-184 were introduced into the pDEST15 vector (Invitrogen) to fuse them to the GST coding sequence for protein expression in *E. coli*; a carboxyl-terminal $(His)_6$ tag was attached to these PALB2 fragments.

PALB2 purification from insect cells

pDEST20 vectors containing full length PALB2, PALB2 1-579, PALB2 611-764, and PALB2 850-1186 were introduced into the *E. coli* strain DH10Bac, and the resulting bacmids were verified by PCR and used to transfect SF9 cells to generate recombinant baculoviruses. The viruses were amplified in SF9 cells, and 4 ml of the amplified viral stock was used to infect 400 ml of Hi5 insect cells. After a 48-h incubation at 27ºC, cells were harvested by centrifugation and quickly frozen at -80°C. All the subsequent steps were carried out at 0-4°C. Extract was prepared by sonication of cell suspension in 30 ml of cell breakage buffer (50 mM Tris-HCl, pH 7.5, 600 mM KCl, 2 mM DTT, 10% sucrose, and the following protease inhibitors: aprotinin, chymostatin, leupeptin, and pepstatin A at 3 μg.ml^{-1} each). After centrifugation (100,000 x *g* for 80 min), the clarified lysate was loaded on a 6 ml Q Sepharose fastflow resin (Amersham) pre-equilibrated with buffer K (20 mM KH_2PO_4, pH 7.4, 0.5 mM EDTA, 1 mM DTT, 10% glycerol, and 0.01% Igepal (Sigma)) containing 250 mM KCl, and the column was developed with a 30 ml, 250-1,000 mM KCl gradient. PALB2 and PALB2 1-579 eluted from the Q Sepharose column at ~400 mM KCl, whereas PALB2 611-764 eluted at ~300 mM KCl and 850-1186 eluted at ~450 mM KCl. Peak fractions were pooled and incubated with 1.5 ml of glutathione-Sepharose 4 beads (GE Healthcare) for 2 h. The beads were washed sequentially with 20 ml of buffer K containing 1 M KCl and 500 mM of KCl, respectively, before eluting the PALB2 species with 6 ml of 25 mM glutathione in buffer K containing 500 mM KCl. The eluate was diluted with an equal volume of 10% glycerol and fractionated in a 1 ml Mono Q column with a 10 ml gradient of 150-800 mM KCl. Fractions containing the peak of PALB2, PALB2 1-579 or PALB2 850-1186 (eluting at ~400 mM KCl; 20 μg, 150 μg or 250 μg total, respectively) or PALB2 611-764, were pooled and concentrated in an Amicon-30 micro-concentrator (Millipore) (to 0.3 mg.ml^{-1} for PALB2, 0.6 mg.ml^{-1} for PALB2 1-579, and 1.2 mg.ml^{-1} for PALB2 611-764 and 850-1,186) and stored in small portions at -80°C.

Purification of GST-tagged PALB2 fragments from *E. coli*

For the purification of the GST- and $(His)_6$-tagged PALB2 amino-terminal protein fragments encompassing residues 1-184, 1-100, and 101-184, plasmids that encode these protein species were introduced into *E. coli* strain BL21 (DE3). Overnight cultures (250 ml) were diluted in 5 L fresh LB and protein expression was induced by the addition of 0.2 mM IPTG and a 16-h incubation at 16°C. All the subsequent steps were carried out

at 0-4°C. The lysate from the *E. coli* cell paste was prepared by sonication in 50 ml of cell breakage buffer containing 0.01% Igepal and clarified by centrifugation (100,000 × *g* for 90 min). The supernatant was mixed with 2 ml of glutathione-Sepharose 4 beads (GE Heathcare) for 2 h. The beads were washed and bound proteins eluted with 10 mM glutathione as above. The eluate was incubated with 1 ml nickel nitrilotriacetic acid-agarose (Qiagen) for 2 h, and the affinity matrix was washed with 5 ml of 20 mM imidazole in buffer K containing 300 mM KCl before eluting the bound proteins with 5 ml of 100 mM imidazole in the same buffer. The eluate was fractionated in a 1-ml macro-hydroxyapatite column (BioRad) with a 20 ml gradient of 50 to 320 mM KH_2PO_4 in buffer K. Peak fractions (200-320 mM KH_2PO_4) were pooled and concentrated in an Amicon-30 micro-concentrator to ~ 5 $mg.ml^{-1}$ protein (total yield of ~0.5 mg of each of the three PALB2 fragments) and stored in small portions at -80°C.

Purification of other recombination proteins

Expression of RAD51AP1, RAD51, and *S. cerevisiae* Rad54 in *E. coli* and their purification followed our published procedures[1,4,5]. *S. cerevisiae* Rad51 protein was expressed in yeast and purified according to our published procedures[6].

DNA binding substrates

The oligonucleotides used in the construction of DNA binding substrates have been described (Supplemental Table S1) and the procedure for substrate preparation was as described[7].

SUPPLEMENTARY REFERENCES

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